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(FILE 'USPAT' ENTERED AT 07:44:34 ON 05 MAR 1999)

L1	1	S	5597797/PN
L2	42	S	IGFI
L3	561	S	IGF (3A) "I"
L4	577	S	L2 OR L3
L5	0	S	L4 AND THERAPUTIC
L6	1	S	PHARMACEUT? AND L1
L7	1	S	L6 AND L4
L8	1	S	CITRATE AND BUFFER AND L7
L9	0	S	PHOSPHAT AND BUFFER AND L8
L10	0	S	PHOSPATE AND BUFFER AND L8
L11	1	S	BUFFER AND L8
L12	0	S	GUANIDINE AND L11
L13	1	S	ARGININE AND L8
L14	1	S	(MAKING OR PREPARING) AND L11

=> d l14 kwic

US PAT NO: **5,597,797** [IMAGE AVAILABLE] L14: 1 of 1

ABSTRACT:

A . . . This method involves administering to the mammal an effective amount of growth hormone in combination with an effective amount of **IGF-I**. Preferably, the growth hormone is given so as to have a maintained, continual therapeutically effective presence in the blood, such. . .

SUMMARY:

BSUM(7)

While . . . subjects to release GH is due to the feedback inhibition operated by the elevated plasma levels of insulin-like growth factor (**IGF-I**) (Loche et al., Clin. Endocrinol., 27: 145-153 [1987]), in fact, no correlation was found between **IGF-I** and indices of overweight. Cordido et al., Horm. Res., 36: 187-191 (1991). Thus, adiposity is not associated with a decline in **IGF-I** levels. Hochberg et al., Metabolism, 41: 106-112 (1992); Gama et al., Clin. Chim. Acta, 188: 31-38 (1990); Rosskamp et al.,. . . 48-50 (1987). Further, impaired hGH stimulation in obese human subjects is not explained by an altered relationship between hGH and **IGF-I** levels. Jungmann et al., Med. Klin., 86: 237-240 (1991). Nor does reduction in circulating insulin levels lead to a higher. . .

SUMMARY:

BSUM(11)

IGF-I production is under the dominant stimulatory influence of GH, and some of the **IGF-I** binding proteins are also influenced by GH. See Tanner et al., Acta Endocrinol., 84: 681-696 (1977); Uthne et al., J. Clin. Endocrinol. Metab., 39: 548-554 (1974). For general reviews of **IGF-I**, see Baxter, Advances in Clinical Chemistry, 25: 49 (1986); Clemmons and Underwood, Clinics in Endocrin. and Metab., 15: 629

(1986). The use of **IGF-I** and GH by injection to produce weight gain and to have anabolic and growth-promoting effects in mammals, including diabetic patients,. . .

SUMMARY:

BSUM(17)

IGF-I is reported to lower blood glucose levels in rats and humans for use in treating diabetes and the secondary effects. . . et al., J. Endocrin., 122: 661-670 (1989); Zenobi et al., J. Clin. Invest., 89: 1908-1913 (1992). In contrast to GH, **IGF-I** and insulin have a known anti-lipolytic effect. Zapf et al., J. Clin. Invest., 77: 1768-1755 (1986); Guler et al., N.. . . al., Diabetes, 39: 340-347 (1990). Further, it has been observed that obese Zucker rats are resistant to the effects of **IGF-I** and insulin on glucose and amine acid metabolism. Jacob et al., Diabetes, 41: 691-697 (1992).

SUMMARY:

BSUM(18)

The most recent study of the effect of **IGF-I** on body composition was by Certain et al. (Endocrinology, 130: 2924-2930 [1992]), who injected recombinant human **IGF-I** (three times a day at 150 .mu.g/kg/day for 8 weeks) in castrate male sheep fed a pelleted and lucerne chaff diet. Treatment caused plasma **IGF-I** levels to rise, plasma insulin to fall, and tibia, spleen, and kidney weights to increase. However, despite **IGF-I** having obvious efficacy, it had no detectable effect on body fat. These authors state that their results are consistent with. . . Endocrinol., 124: 151-158 [19901]) showing similar body composition at equal body weights in mice selected for high and low plasma **IGF-I** concentrations. They conclude that the effects of GH on reducing body fat are not mediated solely through circulating **IGF-I**.

SUMMARY:

BSUM(19)

In . . . state was induced in young rats by diabetes, dexamethasone, or intestinal resection, and then the catabolic animals were treated with **IGF-I** or **IGF-I** analogues. Ballard et al., in Modern Concepts of Insulin-Like Growth Factors, ed. Spencer, p. 617-627 (1991). The authors reported that. . .

SUMMARY:

BSUM(20)

In . . . (Guler et al., Acta Endo., 121: 456-464 [1990]), mini-poodles were treated for 130 days with 6 mg/day of recombinant human **IGF-I**. There was no change in overall body growth but there was a reduced body mass index, which the authors suggest might have been caused by **IGF-I**. However, they state that this suggestion is to be interpreted with great caution, and that recombinant human **IGF-I** may well alter carbohydrate and lipid metabolism in the opposite direction of GH.

SUMMARY:

BSUM(21)

In the hypophysectomized rat, **IGF-I** treatment, at doses that caused a large increase in body and organ weights, had no effect on the

chemical composition of the skin or carcass. In particular, the percentage of fat was not changed by **IGF-I** treatment. Clark and Cronin, Abstract D8, 2nd International IGF Symposium, San Francisco, Calif., 1991.

SUMMARY:

BSUM(22)

In a recent review summarizing the accumulated knowledge at that time of insulin and **IGF-I** activity on different tissues (Froesch et al., TEM, 254-260 [May/June 1990]), it is stated on page 256 that small doses of **IGF-I** may be expected not to affect adipose tissues and this was observed in the rat. They also state that **IGF-I** administration to the rat in vivo had much more marked effects on muscle than on adipose tissue, citing Zapf et al., J. Clin. Invest., 77: 1768 [1986]. In humans, they state that, compared to insulin, the hypoglycemic potential of **IGF-I** is relatively greater than its anti-lipolytic potential, citing Guler et al., N. Engl. J. Med., 317: 137 [1987].

SUMMARY:

BSUM(23)

It . . . Can. J. Biochem. Physiol., 35: 913 [1957]), food intake in young non-obese dwarf rats was unaffected by either GH or **IGF-I** infusions. Skottner et al., Endocrinology, 124: 2519-2526 (1989).

SUMMARY:

BSUM(24)

Data . . . as frequent intermittent injections of GH. However, it has been disclosed that infusions of GH, alone or in combination with **IGF-I**, in amounts that maintain a continuous effective plasma GH concentration, are necessary to stimulate the immune system (GH-responsive lymphoid tissues). . .

SUMMARY:

BSUM(31)

Accordingly, . . . a method for treating obesity or preventing obesity in a mammal comprising administering to the mammal an effective amount of **IGF-I** and GH. The GH is optimally administered such that its therapeutically effective concentration is maintained continuously in the blood of. . .

DRAWING DESC:

DRWD(3)

FIG. 2 shows the serum **IGF-I** levels after 8 days of treatment for the rats treated as described in FIG. 1, where the key is given. .

DRAWING DESC:

DRWD(12)

FIG. . . . lines), hGH 100 .mu.g pump (intermediate shading), hGH 300 .mu.g injection (narrow diagonal lines), hGH 100 .mu.g injection (horizontal lines), **IGF-I** (dark shading), hGH pump/**IGF-I** (solid bar), hGH injection/**IGF-I** (light shading), PEG-GH (wide diagonal lines), and lean control (very light shading).

DRAWING DESC:

DRWD(13)

FIG. . . . cumulative daily body weight changes over 14 days treatment in obese female dw/dw rats. The groups were: control (open squares), **IGF-I** (solid squares), GH by daily injection (open circles), GH infusion (solid circles), and GH infusion plus **IGF-I** (solid squares).

DRAWING DESC:

DRWD(18)

FIG. 17 shows the serum **IGF-I** levels after 14 days in the ten groups of female dw/dw rats, where the key is described in the legend. .

DRAWING DESC:

DRWD(21)

FIG. 20 shows the effect of **IGF-I**, GH and a combination of GH and **IGF-I** on fat mass when administered subcutaneously to AIDS patients. The solid black bars indicate placebo, the bars with a black background having white slashes indicate GH, the speckled bars indicate **IGF-I**, and the bars with a white background having black slashes indicate GH and **IGF-I**. The number of patients treated per group are indicated by the N numbers below the bar graphs, and the y. . .

DETDESC:

DETD(11)

As used herein, "**IGF-I**" refers to insulin-like growth factor from any species, including bovine, ovine, porcine, equine, arian, and preferably human, in native-sequence or in variant form, and from any source, whether natural, synthetic, or recombinant. **IGF-I** has been isolated from human serum and produced recombinantly. See, e.g., EP 123,228 and 128,733.

DETDESC:

DETD(12)

Preferred herein for animal use is that form of **IGF-I** from the particular species being treated, such as porcine **IGF-I** to treat pigs, ovine **IGF-I** to treat sheep, bovine **IGF-I** to treat cattle, etc. Preferred herein for human use is human native-sequence, mature **IGF-I**, more preferably without a N-terminal methionine, prepared, e.g., by the process described in EP 230,869 published Aug. 5, 1987; EP 128,733 published Dec. 19, 1984; or EP 288,451 published Oct. 26, 1988. More preferably, this native-sequence **IGF-I** is recombinantly produced and is available from Genentech, Inc., South San Francisco, Calif. for clinical investigations. Also preferred for use is **IGF-I** that has a specific activity greater than about 14,000 units/mg as determined by radioreceptor assay using placenta membranes, such as. . .

DETDESC:

DETD(13)

The preferred **IGF-I** variants are those described in U.S. Pat.

No. 5,077,276 issued Dec. 31, 1991, in PCT WO 87/01038 published Feb. 26,. . . the N-terminus. The most preferred variant has the first three amino acids from the N-terminus deleted (variously designated as brain **IGF**, tIGF-**I**, des(1-3)-**IGF**-**I**, or des-**IGF**-**I**).

DETDESC:

DETD(16)

The GH in combination with **IGF**-**I** is directly administered to the mammal by any suitable technique, including parenterally, intranasally, orally, or by absorption through the skin.. . . on the medical history of the patient, including any perceived or anticipated side or reduced anabolic effects using hGH or **IGF**-**I** alone. Examples of parenteral administration include subcutaneous, intramuscular, intravenous, intraarterial, and intraperitoneal administration.

DETDESC:

DETD(17)

The GH and **IGF**-**I** are administered so as to be in effective amounts. The GH may be administered non-continuously, such as at particular times. . .

DETDESC:

DETD(25)

The . . . about pH 5-9, more preferably 7-9 if the reactive groups on the GH are lysine groups. Generally, the process involves **preparing** an activated polymer (with at least one terminal hydroxyl group), **preparing** an active substrate from this polymer, and thereafter reacting the GH with the active substrate to produce the GH suitable. . .

DETDESC:

DETD(29)

PEGylation . . . the total lysine concentration of hGH to a solution containing 2 mg/ml of hGH in 50 mM of sodium borate **buffer** at pH 8.5 or PBS at pH 7, and mixing at room temperature for one hour. Products are separated on. . .

DETDESC:

DETD(30)

PEGylation of the cysteine mutants of hGH with PEG-maleimide is accomplished by **preparing** a single cysteine mutant of hGH by site-directed mutagenesis, secreting it from an E. coli 16C9 strain (W3110 delta tonA. . . made by reacting monomethoxyPEG amine with sulfo-MBs in 0.1M sodium phosphate pH 7.5 for one hour at room temperature and **buffer** exchanged to phosphate **buffer** pH 6.2. Next hGH with a free extra cysteine is mixed in for one hour and the final mixture is. . .

DETDESC:

DETD(32)

A preferred manner of **making** PEG-hGH, which does not contain a cleavable ester in the PEG reagent, is described as follows: Methoxypoly(ethylene glycol) is converted. . .

DETDESC:

DETD(33)

The . . . reagent is then reacted with 12 mg/mL of GH using a 30-fold molar excess over GH in a sodium borate **buffer**, pH 8.5, at room temperature for one hour and applied to a Q Sepharose column in Tris **buffer** and eluted with a salt gradient. Then it is applied to a second column (phenyl Toyopearl) equilibrated in 0.3M sodium **citrate buffer**, pH 7.8. The PEGylated hGH is then eluted with a reverse salt gradient, pooled, and **buffer**-exchanged using a G25 desalting column into a mannitol, glycine, and sodium phosphate **buffer** at pH 7.4 to obtain a suitable formulated PEG7-hGH.

DETDESC:

DETD(34)

The . . . gel electrophoresis in 10% SDS is appropriately run in 10 mM Tris-HCl pH 8.0, 1 00 mM NaCl as elution **buffer**. To demonstrate which residue is PEGylated, tryptic mapping can be performed. Thus, PEGylated hGH is digested with trypsin at the. . .

DETDESC:

DETD(38)

The **IGF-I** may be administered by any means, including injections (single or multiple, e.g., 1-4 per day) or infusions. As with the GH, the **IGF-I** may be formulated so as to have a continual presence in the blood during the course of treatment, as described. . .

DETDESC:

DETD(39)

In addition, the **IGF-I** is appropriately administered together with any one or more of its binding proteins, for example, those currently known, i.e., IGFBP-1, IGFBP-2, IGFBP-3, IGFBP-4, IGFBP-5, or IGFBP-6. The **IGF-I** may also be coupled to a receptor or antibody or antibody fragment for administration. The preferred binding protein for **IGF-I** herein is IGFBP-3, which is described in WO 89/09268 published Oct. 5, 1989 and by Martin and Baxter, J. Biol.. . .

DETDESC:

DETD(40)

The administration of the IGF binding protein with **IGF-I** may be accomplished by the method described in U.S. Pat. No. 5,187,151, the disclosure of which is incorporated herein by reference. Briefly, the **IGF-I** and IGFBP are administered in effective amounts by subcutaneous bolus injection in a molar ratio of from about 0.5:1 to. .

DETDESC:

DETD(41)

Preferably, the administration of both **IGF-I** and GH is by continuous infusion using, e.g., intravenous or subcutaneous means. More preferably, the administration is subcutaneous for both **IGF-I** and GH.

DETDESC:

DETD(42)

The GH in combination with **IGF-I** to be used in the therapy will be formulated and dosed in a fashion consistent with good medical practice, taking into account the clinical condition of the individual patient (especially the side effects of treatment with GH or **IGF-I** alone or growth retardation after continuous GH treatment), the site of delivery of the **IGF-I** and GH composition(s), the method of administration, the scheduling of administration, and other factors known to practitioners. The "effective amounts". . . amounts that reduce the obesity of a subject over the reduced obesity that is obtained using the same amount of **IGF-I** or GH individually or prevent obesity or obesity-related conditions from occurring in the first place.

DETDESC:

DETD(43)

As a general proposition, the total **pharmaceutically** effective amount of each of the **IGF-I** and GH administered parenterally per dose will be in the range of about 1 .mu.g/kg/day to 10 mg/kg/day of patient. . . 0.01 mg/kg/day, and most preferably for humans between about 0.01 and 1 mg/kg/day for each hormone. If given continuously, the **IGF-I** and GH are each typically administered at a dose rate of about 1 .mu.g/kg/hour to about 50/.mu.g/kg/hour, either by 1-4. . .

DETDESC:

DETD(44)

It is noted that practitioners devising doses of both **IGF-I** and GH should take into account the known side effects of treatment with these hormones. For hGH the side effects. . . al., J. Clin. Endocrinol. Metab., 21: 361-370 [1961]), as well as hyperinsulinemia and hyperglycemia. The major apparent side effect of **IGF-I** is hypoglycemia. Guler et al., Proc. Natl. Acad. Sci. USA, 86: 2868-2872 (1989). Indeed, the combination of **IGF-I** and GH may lead to a reduction in the unwanted side effects of both agents (e.g., hypoglycemia for **IGF-I** and hyperinsulinism for GH) and to a restoration of blood levels of GH the secretion of which is suppressed by **IGF-I**.

DETDESC:

DETD(45)

For parenteral administration, in one embodiment, the **IGF-I** and GH are formulated generally by mixing each at the desired degree of purity, in a unit dosage injectable form (solution, suspension, or emulsion), with a **pharmaceutically** acceptable carrier, i.e., one that is non-toxic to recipients at the dosages and concentrations employed and is compatible with other. . .

DETDESC:

DETD(46)

Generally, the formulations are prepared by contacting the **IGF-I** and GH each uniformly and intimately with liquid carriers or finely divided solid carriers or both. Then, if necessary, the. . .

DETDESC:

DETD(47)

The . . . chemical stability. Such materials are non-toxic to recipients at the dosages and concentrations employed, and include buffers such as phosphate, **citrate**, succinate, acetic acid, and other organic acids or their salts; antioxidants such as ascorbic acid; low molecular weight (less than. . .

DETDESC:

DETD(48)

The **IGF-I** and GH are each typically formulated individually in such vehicles at a concentration of about 0.1 mg/ml to 100 mg/ml, preferably 1-10 mg/ml, at a pH of about 4.5 to 8. Full-length **IGF-I** is generally stable at a pH of no more than about 6; des(1-3)-**IGF-I** is stable at about 3.2 to 5; hGH is stable at a higher pH of, e.g., 7.4-7.8. It will be understood that use of certain of the foregoing excipients, carriers, or stabilizers will result in the formation of **IGF-I** or GH salts.

DETDESC:

DETD(49)

In addition, the **IGF-I** and GH, preferably the full-length **IGF-I**, may be formulated together in an appropriate carrier vehicle to form a **pharmaceutical** composition that preferably does not contain cells. In one embodiment, the **buffer** used for formulation will depend on whether the composition will be employed immediately upon mixing or stored for later use. If employed immediately after mixing, a mixture of full-length **IGF-I** and GH can be formulated in mannitol, glycine, and phosphate, pH 7.4. If this mixture is to be stored, it is formulated in a **buffer** at a pH of about 6, such as **citrate**, with a surfactant that increases the solubility of the GH at this pH, such as 0. 1% polysorbate 20 or. . .

DETDESC:

DETD(50)

IGF-I and GH to be used for therapeutic administration are preferably sterile. Sterility is readily accomplished by filtration through sterile filtration membranes (e.g., 0.2 micron membranes). Therapeutic **IGF-I** and GH compositions generally are placed into a container having a sterile access port, for example, an intravenous solution bag. . .

DETDESC:

DETD(51)

The **IGF-I** and GH ordinarily will be stored in unit or multi-dose containers, for example, sealed ampoules or vials, as an aqueous. . . reconstitution. As an example of a lyophilized formulation, 10-ml vials are filled with 5 ml of sterile-filtered 1% (w/v) aqueous **IGF-I** and GH solutions, and the resulting mixture is lyophilized. The infusion solution is prepared by reconstituting the lyophilized **IGF-I** and GH using bacteriostatic Water-for-Injection.

DETDESC:

DETD(52)

The GH and **IGF-I** treatment may occur without, or may be imposed

with, a dietary restriction such as a limit in daily food or. . .

DETDESC:

DETD(53)

In addition, the GH and **IGF-I** are appropriately administered in combination with other treatments for combatting or preventing obesity. Substances useful for this purpose include, e.g.,. . .

DETDESC:

DETD(54)

These adjunctive agents may be administered at the same time as, before, or after the administration of GH and **IGF-I** and can be administered by the same or a different administration route than the GH and **IGF-I** are administered.

DETDESC:

DETD(60)

The . . . rat. In addition, it was of interest to discover if obesity was then associated with the induction of insulin and/or **IGF-I** resistance. The obese dw/dw rat might then serve as a model of human disease, especially of Type II diabetes, which. . .

DETDESC:

DETD(61)

The . . . the ob/ob. Mayer et al., Endocrinology, 52: 54-61 (1953). The dw/dw rat has low blood GH and therefore low blood **IGF-I** so it is potentially a good animal model to study the effects of GH and **IGF-I**. Skottner et al., 1989, supra.

DETDESC:

DETD(90)

In . . . phosphate, pH 7.4) at a dose of 240 .mu.g/day, s.c. in three different regimes, or given hGH excipient (the mannitol **buffer** without hGH). For a given dose of GH, the GH given as two injections per day had the largest anabolic effect, continuous infusions being the next most effective treatment, with daily injections having the smallest anabolic effect. The serum **IGF-I** levels in these young rats (FIG. 2) taken 24 hours (once daily injections) or 16 hours (twice daily injections) after. . .

DETDESC:

DETD(91)

Therefore, . . . gains (FIG. 1) were obtained with infusions or twice daily injections of GH and were accompanied by increases in serum **IGF-I**.

DETDESC:

DETD(96)

In addition, Table II shows that GH infusions increased serum GHBP and **IGF-I** concentrations, compared to GH injections. Serum glucose was unchanged but serum cholesterol and triglyceride concentrations were

greatly increased by GH. . .

DETDESC:

DETD(98)

. . .
Treated with Excipient,
hGH (500 .mu.g, s.c.) by Daily Injection,
or by Infusion

Group	Serum IGF-I (ng/ml)	Serum GHBP (ng/ml)	Serum Glucose (mg/dl)	Serum Cholesterol (mg/dl)	Serum Triglyceride (mg/dl)
Excipient	154 .+-..	.	.		

DETDESC:

DETD(128)

The serum **IGF-I**, GHBP, glucose, cholesterol, and triglyceride levels in dw/dw rats at sacrifice are shown in Table IV. Serum **IGF-I** levels in the obese rats were increased by daily GH injection but unchanged by infusions or twice daily injections of. . .

DETDESC:

DETD(129)

It . . . stores. In obese rats (Table III) either weight gain or weight loss was observed. In non-obese rats, hGH infusion increased **IGF-I**, GHBP, cholesterol, and triglyceride levels compared to hGH injection (Table II). But in obese rats **IGF-I** and glucose levels were decreased by GH infusion compared to GH injection, and no rise in GHBP, cholesterol, or triglyceride. . .

DETDESC:

DETD(131)

. . .
hGH (500 .mu.g, s.c.)
by Either Infusion or Once or Twice Daily Injections

Group	Serum IGF-I (ng/ml)	Serum GHBP (ng/ml)	Serum Glucose (mg/dl)	Serum Cholesterol (mg/dl)	Serum Triglyceride (mg/dl)
Excipient	143 .+-..	.	.		

DETDESC:

DETD(139)

The large serum **IGF-I** response to GH infusion seen in non-obese rats was absent in the obese rats. In addition, the serum GHBP levels were not increased by GH infusion. Therefore, there appears to be a degree of GH resistance (using **IGF-I** and serum GHBP as markers) in the obese rat when GH is given continually. However, the lipolytic response to GH. . .

DETDESC:

DETD(149)

The reagents employed were NUTROPIN.RTM. brand hGH, 5-mg vial, and hGH excipient (the **buffer** used in NUTROPIN.RTM. brand hGH, 5 mg/ml equivalent).

DETDESC:

DETD(164)

Use of GH, **IGF-I**, or GH and **IGF-I** to Treat Obese Rats

DETDESC:

DETD(166)

This . . . and to study their dose dependence. In addition, the study was performed to discover the effects of the administration of **IGF-I** alone or its co-administration with GH on carcass composition, whole body and organ weights, serum chemistries, and endocrine hormone levels. . .

DETDESC:

DETD(172)

The . . . a 30-fold molar excess over hGH to a solution containing 12 mg/mL of hGH in 50 mM of sodium borate **buffer** at pH 8.5, and the solution was mixed at room temperature for one hour. The reaction mixture was then applied to a Q Sepharose (Pharmacia) column in 30 mM Tris **buffer**, pH 7.8, and eluted with a NaCl gradient. Then it was applied to a phenyl Toyopearl 650S column equilibrated in 0.3M sodium **citrate buffer**, pH 7.8. The PEGylated hGH was eluted from the column with a reverse salt gradient from 0.3 molar sodium **citrate**, pH 7.8, to 0 molar sodium **citrate** and the fractions containing PEGylated hGH of the appropriate size were pooled. The pool was then **buffer**-exchanged using a G25 desalting column into a **buffer** containing 0.25M mannitol, 0.02M glycine, and 5 mM sodium phosphate, pH 7.4, so as to have a concentration of 1.75 mg/mL. The PEG7-hGH was diluted further in the mannitol **buffer** so as to have a final concentration of 1 mg/mL when used in the rats for this study.

DETDESC:

DETD(173)

The recombinant human GH was NUTROPIN.RTM. brand hGH, 5-mg vial. Recombinant human **IGF-I** [available commercially from KabiGen AB, Stockholm, Sweden (specific activity>14,000 U/mg by radioreceptor assay using placental membranes) or available for clinical investigations from Genentech, Inc., South San Francisco] was employed in all the **IGF-I** experiments detailed in the examples. For this example, the **IGF-I** was dissolved at 18 mg/ml in 10 mM **citrate buffer** and 126 mM NaCl, pH 6.0, while for hGH the excipient was 5 mM phosphate

buffer.

DETDESC:

DETD(176)

Experimental Design:*

Group	Daily Injection	hGH pump	**IGF-I** pump
1	excipient	excipient	excipient
2	excipient	hGH 300 .mu.g	excipient
3	excipient	hGH 100 .mu.g	excipient
4	hGH 300 .mu.g	excipient	excipient
5	hGH 100 .mu.g	excipient	excipient
6	excipient	excipient	**IGF-I** 216 .mu.g
7	excipient	hGH 300 .mu.g	**IGF-I** 216 .mu.g
8	hGH 300 .mu.g	excipient	**IGF-I** 216 .mu.g
9	PEG7-hGH 100 .mu.g	excipient	excipient
lean 10	--	--	--

*The doses given. . .

DETDESC:

DETD(177)

On . . . 70% isopropyl alcohol swab. A small subcutaneous incision was made dorsally and two ALZA 2002 osmotic mini-pumps containing either hGH, **IGF**-I, or excipient were placed subcutaneously. The wound was closed using 9-mm autoclips.

DETDESC:

DETD(179)

On . . . for histology. Serum chemistries were measured using a Monarch clinical chemistry analyzer. Serum insulin was measured by radioimmunoassay. Serum total **IGF**-I also was measured by radioimmunoassay, after the samples were extracted using acid/ethanol.

DETDESC:

DETD(183)

FIG. . . . state with a lack of weight gain or loss (1.5.+-.9.0 g). After 14 days of treatment the hGH infusion and **IGF-I** combination showed a very consistent and very severe catabolic effect, with the average weight loss over 14 days being -50.2.+-.10.0. . . was significantly greater than ($p<0.05$), and over twice, that in the group receiving the 300-.mu.g hGH infusion alone (-23.8.+-.31.1 g). **IGF-I** had no significant effect on weight gain, although at 14 days weight gain occurred (10.0.+-.4.5 g) rather than weight loss.. . .

DETDESC:

DETD(184)

FIG. . . . shows the dramatic differences between the two hGH regimes

and between the effect of GH alone and GH given with **IGF-I**.

DETDESC:

DETD(187)

B. . . . increase absolute kidney weight, and dramatically increased relative kidney weight compared to controls or animals treated with GH by injection. **IGF-I**-treated rats had significantly larger kidneys than those of the obese controls but were no different statistically from those of the. . .

DETDESC:

DETD(188)

C. . . . Rats receiving the 300-.mu.g infusions of hGH had significantly larger livers relative to body weight than those of the controls. **IGF-I** had no statistical effect when compared to control and had no additive effect when given in combination with hGH when. . .

DETDESC:

DETD(189)

D. Spleen weight: Spleen weights of rats receiving **IGF-I** alone were significantly larger than those receiving **IGF-I** in combination with hGH infusions. This suggests that the spleen growth response to **IGF-I** was blocked by hGH infusion. These data were not expected, as effects of **IGF-I** so dramatically blocked by hGH infusion had not previously been seen.

DETDESC:

DETD(190)

E. . . . injections at either 100 or 300 .mu.g/day had no change in the absolute weight of the retroperitoneal or gonadal depots. **IGF-I** alone and PEG7-hGH had no significant effect on fat-pad mass.

DETDESC:

DETD(191)

IGF-I infusion when given in combination with hGH injections lost significantly ($p<0.05$) more fat-pad mass than that of control, and the largest effect on body composition was that of combined GH infusions and **IGF-I** infusions. In this latter group the adipose mass was dramatically reduced to that of control grain-fed rats, and was reduced. . .

DETDESC:

DETD(193)

1. . . . ANOVA insulin levels were not statistically different overall. See FIG. 16. But it should be noted that in the hGH infusion/**IGF-I** combination treatment group most animals had insulin concentrations that were.ltoreq.0.2 ng/ml, which is the minimum detectable level for this assay. It therefore appeared that **IGF-I** reduced insulin levels and that GH infusions plus **IGF-I** infusions reduced the insulin levels even further.

DETDESC:

DETD(194)
2. Serum **IGF-I**: Serum **IGF-I** concentrations in the obese rats were not affected by GH infusions (confirming the data in Table IV). See FIG. 17. As might be expected, **IGF-I** infusions increased serum **IGF-I** concentrations. But these concentrations were decreased by GH co-administration.

DETDESC:

DETD(195)
3. Glucose: Glucose levels were dramatically lower in the hGH/**IGF-I** infusion group (59.6 mg/dl.+-.6.3) as were the glucose levels of the high-dose hGH infusion group (98.2 mg/dl.+-.48.9). See FIG. 18.. . .

DETDESC:

DETD(196)
4. Triglycerides: The serum triglycerides were significantly reduced when hGH and **IGF-I** were infused in combination. Infusions of **IGF-I** or hGH alone had no significant effect on serum triglycerides. Daily injections of hGH significantly increased serum triglycerides; however, when **IGF-I** was infused in combination with hGH injections, serum triglycerides were not statistically different from that of control. See FIG. 18.. . .

DETDESC:

DETD(198)
6. . . . were maintained on a high-fat diet so the thin rats were eating more protein relative to calories. The groups receiving **IGF-I** have lower mean BUNs than the groups receiving hGH alone.

DETDESC:

DETD(199)
7. Calcium: Calcium was increased by hGH infusion and by PEG7-hGH but decreased if **IGF-I** was given alone or in combination with hGH. See FIG. 19.

DETDESC:

DETD(203)

In addition, **IGF-I** was employed in combination with hGH to determine if **IGF-I** would antagonize the lipolytic effect of the hGH infusions. Surprisingly it was found that the combination was even more effective. . .

DETDESC:

DETD(204)

In the non-obese animal **IGF-I** infusions and GH injections have an additive anabolic effect, but **IGF-I** combined with GH infusions does not have an additive anabolic effect. See U.S. Pat. No. 5,126,324, supra. It was therefore unexpected that the combination of **IGF-I** and GH (**IGF-I** infusions and GH infusions or GH injections) induced a lipolytic effect in obese mammals. Particularly surprising was the dramatic synergistic effect of GH infusion and **IGF-I** infusion on weight loss and on adipose tissue mass that occurred in obese rats.

DETDESC:

DETD(205)

Example . . . dw/dw rats became insulin resistant. GH is considered "diabetogenic," i.e., causing insulin resistance. Therefore, the administration of GH especially with **IGF-I** to an obese animal, where blood glucose falls (rather than rises) and insulin falls (rather than rises) is contrary to. . .

DETDESC:

DETD(206)

From . . . be expected to restore insulin sensitivity, so that in obese humans receiving insulin treatment, the appropriate administration of GH and **IGF-I** as described herein would allow insulin administration to be reduced or stopped. Therapy with GH and **IGF-I** therefore is expected to prevent, or prevent the progression of, human type II diabetes in the obese patient.

DETDESC:

DETD(207)

It . . . protocols and procedures, the veterinarian or clinician will be able to adjust the doses, scheduling, and mode of administration of **IGF-I** and GH and their variants to achieve maximal effects in the desired mammal being treated. Humans are believed to respond. . .

DETDESC:

DETD(209)

Use of GH, **IGF-I**, or GH and **IGF-I** to Treat Human Patients

DETDESC:

DETD(210)

Clinical data were obtained from male AIDS patients with an average age of 39 years comparing control, **IGF-I** alone, GH alone, and GH and **IGF-I** together. In these studies, **IGF-I**, produced and formulated as described in Example III, was administered to the AIDS patients subcutaneously at a dose of 5. . .

DETDESC:

DETD(211)

After . . . the treatment after 12 weeks dropped to 9 for the control group, 6 for the GH group, 4 for the **IGF-I** group, and 6 for the GH and **IGF-I** group.

DETDESC:

DETD(212)

This study showed that after 12 weeks of treatment the combination of **IGF-I** and GH produced an average increase in lean body mass of about 7 lb. with concomitant fat loss. In the most dramatic case the patient gained 3 kg of lean body mass but lost 1 kg of fat. **IGF-I** alone showed no change over placebo, while GH alone showed a small increase in lean body mass without the fat. . . had by far the greatest loss of fat mass, suggesting a synergistic effect on fat loss when the hGH and **IGF-I** are administered together for 12 weeks. A similar effect was observed after six weeks of drug therapy.

DETDESC:

DETD(213)

TABLE V

Fat Mass after 12 Weeks Hormone Treatment	
Placebo .+-. SD*	0.00 .+-. 0.81
GH .+-. SD	0.00 .+-. 0.83
IGF-I .+-. SD	-0.20 .+-. .80
GH + **IGF-I** .+-. SD	-1.80 .+-. 1.94

. . .

CLAIMS:

CLMS(1)

What . . .

method for reducing total body fat mass in an obese mammal comprising administering to the mammal an effective amount of **IGF-I** and growth hormone.

CLAIMS:

CLMS(3)

3. . . . Type II diabetes and the need of the human for insulin is decreased upon the administration of growth hormone and **IGF-I**.

CLAIMS:

CLMS(5)

5. The method of claim 2 wherein the **IGF-I** is human native-sequence, mature **IGF-I**.

CLAIMS:

CLMS(6)

6. The method of claim 4 wherein the **IGF-I** is human native-sequence, mature **IGF-I**.

CLAIMS:

CLMS(7)

7. The method of claim 1 wherein the administration of **IGF-I** is be continuous infusion.

CLAIMS:

CLMS(8)

8. The method of claim 1 wherein the **IGF-I** is in a sustained-release formulation.

CLAIMS:

CLMS(17)

17. the method of claim 1 wherein the effective amount of **IGF-I** is at least 0.01 mg/kg/day.

CLAIMS:

CLMS(18)

18. The method of claim 1 wherein the growth hormone and **IGF-I** are administered separately.

CLAIMS:

CLMS(19)

19. The method of claim 1 wherein the growth hormone and **IGF-I** are administered as a single formulation.

CLAIMS:

CLMS(20)

20. The method of claim 1 wherein the **IGF-I** is administered with an IGF binding protein.